



FCSE FRESHMAN COLLEGE SUMMER EXPERIENCE

HHMI

Is there MITE Polymorphism in Maize Strains B73 vs. Palomero?

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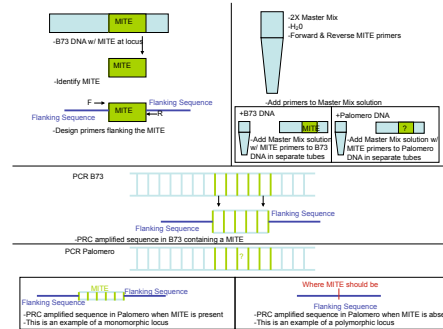
Dynamic Genome Summer 2009



Abstract

Maize is an ideal model system for genetic analysis because of its numerous visual phenotypes (kernel shape and color) and large collection of active transposable elements (T_{es}). Miniature inverted repeat transposable elements (MITEs), are small, non-autonomous elements, that are repeated in high copy numbers throughout the genes of maize. In our experiment, we focused on polymorphism in two diverse strains of maize, B73 and Palomero. A MITE present in B73 but not in Palomero, will result in PCR products of different size when visualized on agarose gels. Such a MITE is said to be polymorphic in the two strains. To design our experiment, we exploited the maize genome browser, which contains the sequence of B73 and its annotation. After completing our experiment, we found that some of the MITEs were polymorphic and others were monomorphic, meaning that the MITE appeared in the genomes of both B73 and Palomero.

Experimental Design



To test if our MITEs were polymorphic in B73 and Palomero, we first used BLAST to locate our MITE in the known B73 sequence. Once found, we needed to design primers that could amplify this region in both B73 and in Palomero. We used the Primer3Plus program to design our primers. We then set up and ran PCR with B73 and Palomero (separate reactions and with either MITE or actin primers) and with only water (no DNA) as a negative control. Actin primers were used to make sure that the DNA was capable of being amplified. We had previously tested these primers and as such, we knew they worked. Thus, actin was a positive control in this experiment. We then visualized the PCR products on agarose gels.

Agarose gel analysis of our PCR data

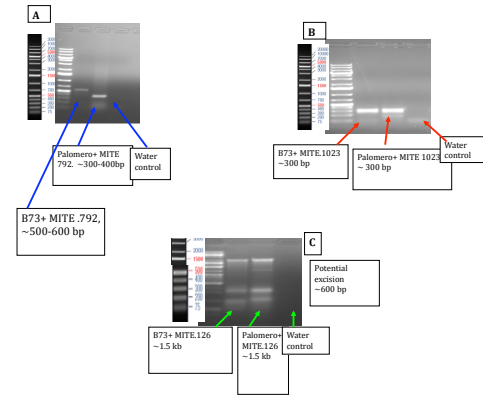
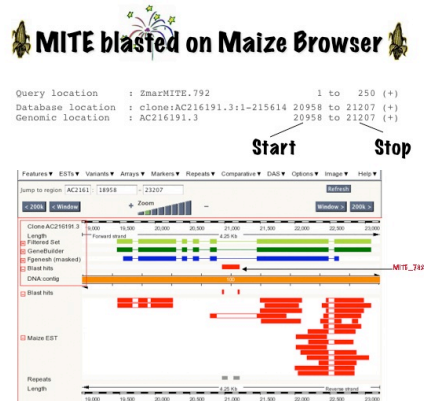
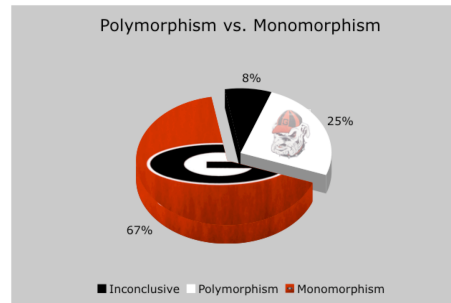


Figure A represents Polymorphism of MITE.792 in B73 and Palomero.
Figure B represents Monomorphism of MITE.1023 in B73 and Palomero
Figure C represents a potential excision of MITE.126 as evidenced by the unspecified PCR product

Use of the Maize Browser



Each MITE was blasted against the B73 genome using the Maize Browser website. The alignment view shows the start and stop positions of the MITE. The contig view shows the browser's prediction of where the MITE was located in the genome. The MITE sequence can be downloaded from the browser and used for primer design. The picture of the browser shown above displays the gene model in lime green, dark green, and blue. The EST's and blast hits are shown in red.



The class collectively ended up with three different results. The majority of the class (67%) had monomorphic results, which means that the MITE was located at the same locus in both Palomero and B-73 genomes. One fourth of the class ended up with polymorphic results, which means their MITE was not present at the same locus in the two strains of maize.

What we learned from this project:

- How to work in a lab— both wet bench and computational
- How to perform PCR, pour and run agarose gels, pipette, extract genomic DNA, design primers for PCR and more...
- How to design experiments including positive and negative controls and using computer databases for annotation
- How to analyze data including agarose gel images, troubleshooting PCR
- “Lab is like a box of chocolates—you never know what you will get.”
- It can actually be fun to work in a lab